49605601

Proposed Protocol to Support Use of EPA Reg. No. 90094-1 with the Mini Chlorine Dioxide System (MCS) to Sterilize/Decontaminate Confined Areas, Specifically Including Biological Safety Cabinets

Test Material
Sodium Chlorite Technical, EPA Reg. No. 90094-1
(Alternate trade name – *"CD Generation Part "A"*)

Data Requirement
OCSPP Guideline 810.2100, Sterilants

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Study Completion Date
To Be Determined

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Laboratory Project / Guideline Number
To Be Determined

PROTOCOL SUMMARY

PURPOSE:

The purpose of this proposed study is to satisfy EPA Guideline 810.2100 as required to support the amendment of EPA Reg. No. 90094-1 to include use as a sterilant in confined spaces when applied using the DRS Laboratories' (DRS's) Mini Chlorine Dioxide System® (MCS) as directed.

BACKGROUND:

The label amendment is to add the use of this product to generate gaseous Chlorine Dioxide (CD) for use in the registrant's MCS to decontaminate enclosed area volumes up to 120 cubic feet to include biological safety cabinets.

Label Claims:

CD Generation Part "A" is for use to generate CD gas used to [decontaminate] [sterilize] [fumigate] non-porous and porous surfaces in sealed enclosures, confined spaces, rooms or areas, or vehicles located in government, industrial, manufacturing, fermentation, commercial and institutional microbiological laboratory settings, including human and animal research facilities and areas, cleanrooms; animal isolation rooms, necropsy suites, pass-throughs, airlocks, decontamination chambers, biological safety cabinets, glove boxes, isolators, incubators, animal cages and devices, laboratory equipment, supply and exhaust filter systems, and HEPA filtered devices.

DRS's CD gas generation system has been validated for use to [decontaminate] [sterilize] [fumigate] enclosures up to 120 cubic feet. Use to [decontaminate] [sterilize] [fumigate] larger enclosures can be done on a case-by-case basis if appropriate biological indicators (BI's) to confirm required performance are included in the treatment process. Uses other than those specified in the appropriate DRS equipment Instruction Manual are not permitted and may not be effective. Review and follow all DRS equipment Instruction Manual instructions and precautions on how to properly utilize this product.

METHOD

TESTING FACILITIES and GLP COMPLIANCE:

DRS Laboratories, Inc.:

The CD decontamination procedure will be performed at the facilities of DRS Laboratories, Inc. using a Baker SterilGard® III Advance Biological Safety Cabinet, Model SG:603 (see attached Operator's Manual), which has a total volume of 78 ft^3 as the representative treatment area. DRS will not be making the claim to have conducted the decontamination process in full compliance with 40 CFR Part 160 as that facility is not a contract research laboratory. However, all efforts will be made to comply with GLP regulations as closely as possible.

Azzur Labs LLC:
Azzur Labs LLC will perform the analyses of the Biological Indicators (BIs) for growth using appropriate media and conditions for the respective BI's and in compliance with GLP regulations.

MATERIALS:

TEST SUBSTANCE:

Sodium Chlorite Technical, EPA Reg. No. 90094-1
(Alternate trade name - *CD Generation Part "A"*)
Ingredients:

- 80% Sodium Chlorite
- 20% Inerts

Three lots of EPA Reg. No. 90094-1 will be tested, one of which will be over 60 days of age. The CD gas produced from the Sodium chlorite is the sterilant used in conjunction with the DRS Laboratories MCS generator to generate and distribute CD gas.

The Sodium Chlorite, EPA Reg. No. 90094-1, is reacted with "Solid Component B", which contains Sodium Bisulfate and a proprietary secondary activator to generate CD gas.

THE DRS MCS APPLICATION SYSTEM INCLUDES:

- Control box with CD Generation, Recirculation, and Scrubbing Blower(s)
- CD dispensing assembly, releasing valves and plumbing
- Charcoal Scrubber
- Supply, Return Recirculation Duct Lines
- Supply, Return Sealing Pane(s)
- Humidifier
- Humidity gauge

TREATMENT CONDITIONS:

Preparation of the area for decontamination is discussed in detail in DRS's MCS Generator's Owner's Manual.

TEMPERATURE:

The optimal temperature range for CD gas generation is 59-104°F (15-40°C). For this proposed study, the decontamination process will be conducted within the narrower and lower temperature range of 59°F to 70°F, which is representative of the least optimal ("worst case") conditions within the acceptable temperature range for CD gas generation.

RELATIVE HUMIDITY:

The optimal relative humidity range for CD gas generation is 60% to 85% RH. For this proposed study, the decontamination process will be conducted within the narrower and lower relative humidity range of 60% to 70% RH, which is representative of the least optimal ("worst case") conditions within the acceptable relative humidity range for CD gas generation.

DIFFERENTIAL PRESSURE:

The optimal pressure range for CD gas generation is consider to be ± 0.005 W.C. or shall be neutral in pressure. To be monitored in all tests.

SOIL LOAD:
The Agency has indicated that the use of a soil load on the BI's is a waived requirement. The BI's will be obtained from a commercial supplier.

OTHER:
All lighting shall be turned off or dark.

BIOLOGICAL INDICATORS:

As per the EPA Guideline OCSPP 810.2100, *Sterilants—Efficacy Data Recommendations* dated September 4, 2012, there is some discretion allowed as to what a sterilant should be tested against. We are recommending the test organisms in the chart because they have been utilized in other EPA field testing publically reported. Further, these BI's are commercially available and used routinely to monitor decontamination procedures in the field.

Test Organism	ATCC #	Carrier type
Bacillus atrophaeus	9372	Cellulose*
Bacillus atrophaeus	9372	S. Steel**
Geobacillus stearothermophilus	7953	Cellulose*
Geobacillus stearothermophilus	7953	S. Steel**

* Cellulose BI's enveloped in pouches of Tyvek on one side and Mylar on the other
** Stainless Steel BI's enveloped in pouches of Tyvek both sides.

Based on the Agency's efficacy study review response (EPA Decision No. 488000) dated September 4, 2014, *Bacillus atrophaeus* (ATCC #9372) and *Geobacillus stearothermophilus* (ATCC #7953) appear to be acceptable test organisms to support a sterility claim for CD gas given testing on both stainless steel and paper carriers for both test organisms. Resiliency test data show that *Bacillus atrophaeus* (ATCC 9372) is the most challenging and reliable spore to use as a biological indicator (BI) of the efficacy of CD as a sporicide (see MRID No. 49320201).

A minimum of Sixty carriers-representing each of two types of surfaces, stainless steel discs (representing hard non-porous surfaces) and cellulose (representing porous surfaces) will be tested using spores of both *Bacillus atrophaeus* (ATCC #9372) and *Geobacillus stearothermophilus* (ATCC #7953) on three test material samples (*Sodium Chlorite Technical*, EPA Reg. No. 90094-1) representing three different batches, one of which will be at least 60 days old (240 carriers per sample; a total of 720 carriers; See Attachment A for the three Certificates of Analysis).

PLACEMENT OF BI's:
HEPA filtered devices such as a biological safety cabinet (BSC) are proposed as representing worse case conditions for an enclosed area being treated; see Attachment A. BIs will be placed inside the BSC on the work surface of the unit and outside the HEPA filter of the BSC.

DETERMINATION OF APPROPRIATE AMOUNT OF CD GAS IN DECONTAMINATION CYCLE:

As indicated above, CD gas is generated by combining *CD Generation Part "A"*, EPA Reg. No. 90094-1, with CD generation packet "B" in water. Absolute precision in the generation of CD is not realistic or obtainable and efforts to generate precisely the minimum amount required for a given situation can result in miscalculation and inadequate CD gas generation. Pre-measured packets of the "A" and "B" components are provided for use in 500 mL of water to generate and maintain CD gas equal to the minimum concentration required for sterilization, 0.13 g/ft^3 up to 0.163 g/ft^3 for the 90 minute treatment period. The validation of these CD gas generation values is presented in MRID No. 49320201.

The table below can be used to determine the number of packets of "A" and "B" appropriate for a given enclosure volume range to ensure adequate CD gas is generated. An equal number of packets of "A" and "B" are always used in combination with 500 mL of tap water.

Volume ft^3 (m^3)	**BSC Size** Width - ft^3 (m^3)	**CD Generation** Chemicals
0(0) to 25 (0.7)	0-2 ft. (0.0-0.6)	1 each of A & B
25 (0.7) to 60 (1.7)	3-4 ft. (0.91-1.22)	2 each of A & B
60 (1.7) to 90 (2.5)	5-6 ft. (1.52-1.83)	3 each of A & B
90 (2.5) to 120 (3.4)	n/a – special	4 each of A & B

Note: There is variation in the amount of CD grams generated dependent upon the number of chemical packets used in relationship to the upper enclosure volume within the above ranges. It was found not to be a linear curve but following the above guideline table results in generation and maintenance of the minimum concentration required for sterilization, 0.13 g/ft^3.

Since the test chamber for this study will be a Baker SterilGard® III Advance Biological Safety Cabinet, Model SG;603, which has a total volume of 78 ft^3, 3 packets each of *CD Generation Part "A"*, EPA Reg. No. 90094-1, and CD generation packet "B" will be used to generate the necessary CD gas for the decontamination/sterilization test. Efforts will be taken to reach and maintain the CD gas concentration as close as possible to 0.13 g/ft^3 trying not to exceed this value.

DECONTAMINATION PROCESS:

A humidity source, humidity meter, and various biological indicators are placed within the enclosure to be decontaminated. The enclosure is then sealed incorporating into the seal a gas inlet and outlet port for use with the MCS. After appropriate humidity (i.e., within 60% to 70% RH) and temperature (i.e., within 59°F to 70°F) are confirmed, CD is produced and released and the decontamination cycle begins.

The test material will be used according to DRS's MCS instructions (see attached). The method of CD gas generation utilizes *CD Generation Part A* in water with addition of *CD Generation Part B*, a solid acid, to generate CD gas. CD gas at a concentration of 0.13g/ft^3 (4.7g/m^3) CD gas will be generated and maintained within the treatment enclosure volume. The duration of the decontamination period will be a fixed time of 90 minutes, which will start when the CD gas concentration reaches 0.13g/ft^3.

The CD gas concentration will be continuous monitored using a CD Photometer Model CDP101, which has the following characteristics:

- Measurement Range of 0.00 to 50 mg/l (0.00 to 18,100 ppm),
- Resolution: < ± 0.05 % of respective measuring range, and
- Repeatability: < ± 0.5 % of respective measuring range.

Measurements will be recorded during the decontamination process at 0, 10, 20, 30, 60, and 90 minutes as well as at 10, 20, and 45 minutes during the final scrubbing cycle.

NEUTRALIZATION (SCRUBBING CYCLE):

After a treatment period of 90 minutes, CD gas is removed from the enclosure using the MCS unit's "scrubbing cycle", which circulates the air in the enclosed area through an activated charcoal filter that captures the CD gas. The air inside the treatment area is sampled using a C16 PortaSens II CD gas detector, which has an accuracy of ± 5% and sensitivity of 1% of sensor module. The CD gas level must be below the OSHA PEL limit of 0.1 ppm for the scrubbing cycle can be considered complete. There should also be no noxious odors present; otherwise, the scrubbing cycle will need to be extended until that issue is eliminated. The scrubbing cycle generally takes about 45 minutes and represents the neutralization of the CD gas on the BIs.

After the scrubbing cycle, the enclosure is disengaged from the MCS unit and the enclosure may be unsealed. At that time, the BI's will be aseptically recovered (will take up to one hour to disassemble the BSC) and delivered to the laboratory in zip lock bags maintained at room temperature within one hour for processing. It is anticipated that Azzur Labs will initiate the analysis of the BI's within two hours of their recovery from the treated BSC.

TESTING OF THE BI'S:

Geobacillus stearothermophilus carriers are incubated at 55°C to 60°C. *Bacillus atrophaeus* carriers are incubated at 30°C to 35°C. Aseptically transfer the biological indicator to a tube of Tryptic Soy Broth (TSB). Transfer all test samples before transferring the positive control. Incubate appropriately. Visually inspect the tubes for turbidity at 24 hours to 4 days. Return the tubes to the appropriate incubator. Visually inspect the tubes for turbidity after at least seven days of incubation.

PERFORMANCE CRITERIA:

Evaluation of sterilant success shall be the product shall kill the test spores on all of the 720 BI's (carriers) without any failures (e.g., growth of test organism after treatment). Test samples and negative controls are clear (negative); positive controls are turbid (positive).

PREPARATION OF CONTROLS:

Population Verification of the Biological Indicators

The population of each lot of biological indicator used in the study will be verified prior to use. The verification will be performed according to the manufacturer's instructions.

Acceptance Criteria: Biological Indicators must yield a population of at least 1.0×10^6.

Quality Control Testing of Media

The sterility and growth promotion capability of the TSB used in testing is verified prior to use. Sterility of the TSB is verified at the incubation temperatures used in testing.
Acceptance Criteria: Media must meet the criteria indicated on the manufacturer's certificate of analysis for growth promotion and sterility.

Test Controls
Two unexposed BIs of each type used in testing are submitted as controls with the test samples from each day. A negative control is included by incubating an unopened tube of the same lot of TSB that was used for the test. The positive and negative controls are tested in the same manner as the test samples.
Acceptance Criteria: For the positive control, both unexposed controls for each lot of biological indicator used must be turbid (positive). For the negative control, the unopened tube of TSB must be clear (negative).

REFERENCES:

The references below may be consulted for additional background information.

(1) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 966.04 Sporicidal Activity of Disinfectants, Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(2) *Official Methods of Analysis of the AOAC International*, Chapter 6, Disinfectants, Official Method 2008.05 Quantitative Three Step Method (Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface), Current edition. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, MD 20877-2417.

(3) *Annual Book of ASTM Standards*, Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides, Designation E 2197. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428, current edition.

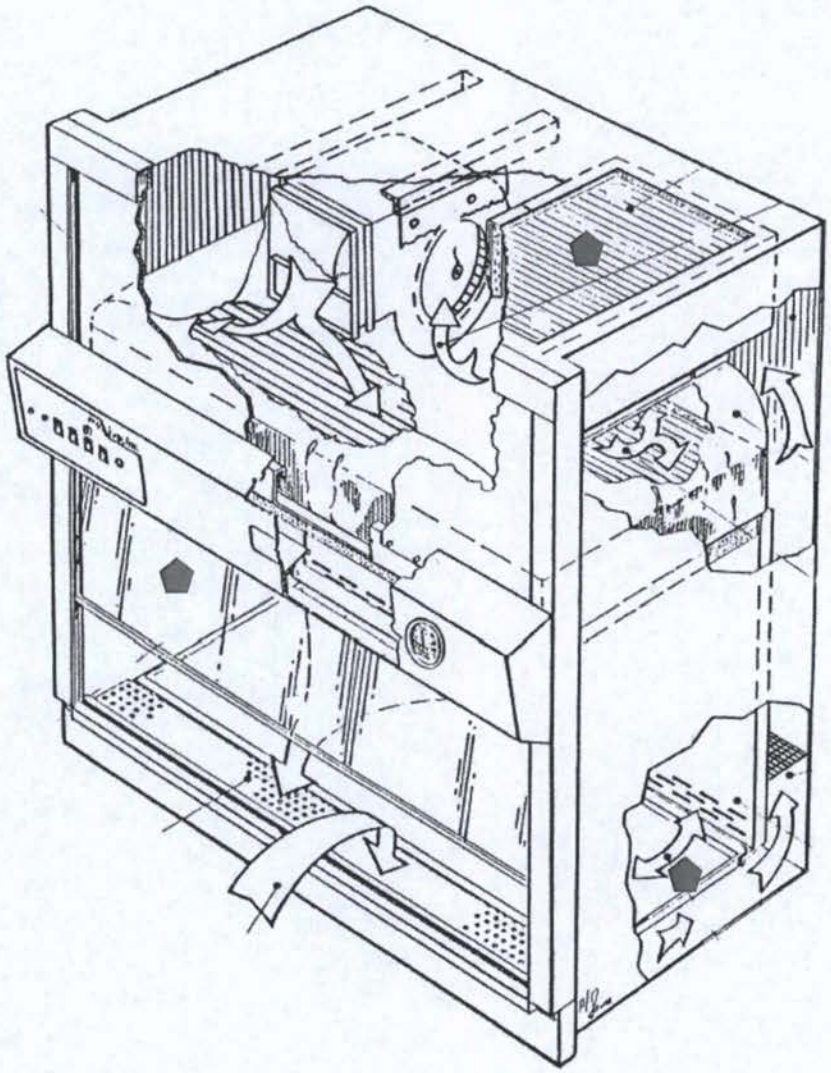
ATTACHMENT A

PLACEMENT AND NUMBER OF THE BIOLOGICAL INDICATORS IN THE BIOLOGICAL SAFETY CABINET

Biological Indicator Placement in the BSC

See figure 1 for BI placement.

1. BI's placed between the pleats on the downstream (clean) side of the exhaust HEPA filter near the center.
2. BI's placed inside the work area of the BSC back or side wall.



Number of *Bacillus atrophaeus* (ATCC 9373) BI's at Each Location in the BSC

Location	Sodium Chlorite, EPA Reg. No. 90094-1 Lot # 1		Sodium Chlorite, EPA Reg. No. 90094-1 Lot # 2		Sodium Chlorite, EPA Reg. No. 90094-1 Lot # 3	
	Porous	Non-Porous	Porous	Non-Porous	Porous	Non-Porous
Exterior - HEPA Filter Center; Clean Side, Between pleats	60	60	60	60	60	60
Internal - Work Area back and side wall	60	60	60	60	60	60
Subtotal carriers	120	120	120	120	120	120
Subtotal carriers	240		240		240	
Total carriers	720					

Number of *Geobacillus stearothermophilus* (ATCC 7953) BI's at Each Location in the BSC

Location	Sodium Chlorite, EPA Reg. No. 90094-1 Lot # 1		Sodium Chlorite, EPA Reg. No. 90094-1 Lot # 2		Sodium Chlorite, EPA Reg. No. 90094-1 Lot # 3	
	Porous	Non-Porous	Porous	Non-Porous	Porous	Non-Porous
Exterior - HEPA Filter Center; Clean Side, Between pleats	60	60	60	60	60	60
Internal - Work Area back and side wall	60	60	60	60	60	60
Subtotal carriers	120	120	120	120	120	120
Subtotal carriers	240		240		240	
Total carriers	720					